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Novel Imaging Techniques, Integrated with Mineralogical, Geochemical and Microbiological Characterization to Determine the Biogeochemical Controls....

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RESULTS TO DATE: Aims and objectives Technetium-99 is a priority pollutant at numerous DOE sites, due to a combination of its long half life (2.1 x 105 years), high mobility as Tc(VII) (TcO4-; pertechnetate anion) in oxic waters, and bioavailability as a sulfate analog. Under anaerobic conditions, however, the radionuclide is far less mobile, forming insoluble Tc(IV) precipitates. As anaerobic microorganisms can reduce soluble Tc(VII) to insoluble Tc(IV), microbial metabolism may have the potential to treat sediments and waters contaminated with Tc. In previous studies we have focused on the fundamental mechanisms of Tc(VII) bioreduction and precipitation, and we have identified direct enzymatic (hydrogenase-mediated) mechanisms, and a range of potentially important indirect transformations catalyzed by biogenic Fe(II), U(IV) or sulfide. These baseline studies have generally used pure cultures of metal-reducing bacteria, in order to develop conceptual models for the biogeochemical cycling of Tc. There is, however, comparatively little known about interactions of metal-reducing bacteria with environmentally relevant trace concentrations of Tc, against a more complex biogeochemical background provided by mixed microbial communities in the subsurface. This information must be available if in situ remediation of Tc(VII) contamination is to be successful at DOE sites. The aim of this project is to use a highly multidisciplinary approach to identify the biogeochemical factors that control the mobility of environmentally relevant concentrations of Tc(VII) in FRC sediments. We will use a combination of geochemical, mineralogical, microbiological and spectroscopic techniques to determine the solubility and phase associations of 99Tc in batch sediment experiments ("progressive microcosms"), where a sequence of terminal electron accepting processes is separated by time. Additional column experiments utilizing FRC sediments containing discrete biogeochemical zones will be challenged with low concentrations of 99mTc, and the mobility of the radionuclide imaged using a -camera. By comparing the pattern of Tc immobilization, with high resolution studies of the mineralogy, geochemistry and microbial ecology of the columns, we will further characterize the biogeochemical controls on Tc mobility in FRC sediments. Column experiments will also utilize similar approaches to determine the stability of immobilized reduced phases of Tc in the presence of oxidizing agents including nitrate. The effectiveness of strategies proposed to stimulate Tc(VII) reduction and precipitation in the subsurface will also be determined in both batch and column experiments. Finally the experimental results will be used to calibrate a modelling approach employing an established coupled speciation and transport code to provide parameters that could potentially be used to make predictions of the mobility of Tc in FRC sediments and other subsurface environments. Objectives and hypotheses The objective of the proposed research is to take a highly multidisciplinary approach to define the biogeochemical factors that control Tc mobility in FRC sediments. The following hypotheses will serve as a guide for the research. These hypotheses will be evaluated with a combination of geochemical, mineralogical, microbiological and modelling approaches, augmented with novel imaging techniques for Tc. These approaches are sufficiently broad in scope that they will yield insights into the biogeochemical factors underlying Tc immobilization, even if the hypotheses themselves are proved to be incorrect. Hypothesis 1. Tc(VII) will be reduced and precipitated in FRC sediments under anaerobic conditions in batch experiments (progressive microcosms). Hypothesis 2. Tc(VII) reduction and precipitation can be visualized in discrete biogeochemical zones in sediment columns using 99mTc and a -camera. Hypothesis 3. Sediment-bound reduced 99mTc can be solublized by perturbations including oxidation by nitrate, and mobilization visualized in real-time using a -camera. Hypothesis 4 The mobility of 99mTc in the sediment columns can be modelled using a coupled speciation and transport code. Results Microcosm-based experiments during the first year of this grant have successfully addressed hypothesis 1 and have given the project a firm scientific foundation. Samples of sediment and groundwater were obtained from the FRC background area (near FW301; depth of 22 to 23 feet) in Fall 2003. The sediments were composed of friable interbedded shale and sandstone, which were mixed under sterile conditions prior to microcosm preparation. Here 16 g of sediment and 34 ml of nitrogen purged FRC background area groundwater

were placed in serum bottles, and the headspace was purged with nitrogen. Samples were prepared in triplicate, amended with acetate and/or nitrate and spiked with a filter sterilized stock solution of pertechnetate to a final concentration of 0.5µM Tc-99. Controls containing no added Tc-99 were also prepared for background scintillation counting, and sterile controls were also prepared in triplicate to check for abiotic interactions of Tc(VII) with the sediment-groundwater matrix. Samples were incubated in the dark at 20°C. Results from these experiments confirmed that the microbial communities in the sediments had the capacity to reduce Fe(III), and that Tc(VII) reduction correlated with ingrowth of Fe(II) in the sediments. Metal reduction was noted both with and without added electron donor (20 mM acetate), suggesting that the sediments contain significant quantities of bioavailable electron donor (e.g. natural organic matter) to support the reduction of Fe(III) and Tc(VII). Molecular and cultivation-dependent analyses of the microbial communities present in the sediments confirmed the presence of Fe(III)-reducing bacteria (including Geobacter species) known to reduce both Fe(III) and Tc(VII) in axenic cultures. Analysis of sediments supplemented with higher concentrations of Tc (0.5 mM), using X-ray absorbance spectroscopy on station 16.5 at the Daresbury synchrotron, confirmed the transformation of Tc(VII) to insoluble Tc(IV) (as hydrous TcO2). Addressing hypothesis 3, we have also initiated studies to quantify the impact of oxidants including nitrate and air on the biogeochemical cycling of technetium in FRC sediments collected from the background area. In the presence of 10 mM nitrate (and 10 mM acetate), there was an increase in the rate and amount of Fe(III)/Tc(VII) reduction in the sediments, possibly indicating that microbial metabolism in the FRC background sediments may be limited by nitrogen. However, additions of 100 mM nitrate, which at high concentrations could potentially compete for electrons during metal reduction, inhibited the reduction of Fe(III) and Tc(VII) completely. New experiments have addressed the impact of high nitrate concentrations on Fe(II) and Tc(IV) in pre-reduced sediments, with a parallel set of experiments ongoing that addresses the impact of aerobic conditions on the stability/solubility of Fe(II) and Tc(IV). Complementary microcosm experiments using low pH/nigh nitrate sediments from Area 3 (near FW 009) are now planned, with the sediment cores already shipped to Manchester. Preliminary column experiments are also being set up to allow the use of 99mTc as a tracer in flow-through experiments to address hypotheses 2 and 3.

DELIVERABLES: Publications Burke, I.T., Lloyd, J.R., Livens, F.R., Boothman, C., Mortimer, R.J.G. and Morris, K. Biogeochemical redox cycling of technetium. Science. submitted. Lloyd, J.R., Anderson, R.T., Macaskie, L.E. (2004) Bioremediation of metals and radionuclides. In "Bioremediation" Ed by R. Atlas and J. Philp. ASM Press Washington (In Press) Lloyd, J.R., Lovley, D.R., Macaskie, L.E. (2004) Biotechnological application of metal-reducing bacteria. Advances in Applied Microbiology. 53: 85-128 J.R. Lloyd and J. C. Renshaw Microbial Transformations of Radionuclides: Fundamental Mechanisms and Biogeochemical Implications in "Biogeochemical Cycles", Vol. 43 of Met. Ions Biol. Syst. (A. Sigel, H. Sigel, and R. K. O. Sigel, eds.), M. Dekker, New York, in press. Invited conference presentations "Microbial reduction of radionuclides" Symposium on Interactions of Heavy Elements with Microorganisms, Tokai, Japan (2004) "Biotechnology and the nuclear fuel cycle" European Meeting for Biotechnologies, Marseille, France (2004) "Microbial reduction of metals and radionuclides of environmental concern" Biometals 2004 Garmisch Partenkirchen, Germany (2004)